Talanta 143 (2015) 145–156

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Freshness estimation of intact frozen fish using fluorescence spectroscopy and chemometrics of excitation–emission matrix

Gamal ElMasry [a,b,*], Hiroto Nagai [a], Keisuke Moria [c], Naho Nakazawa [c], Mizuki Tsuta [d], Junichi Sugiyama [d], Emiko Okazaki [c], Shigeki Nakauchi [a]

[a] Department of Computer Science and Engineering, Toyohashi University of Technology, Toyohashi, Aichi, Japan
[b] Suez Canal University, Faculty of Agriculture, Agricultural Engineering Department, Ismailia, Egypt
[c] Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, Japan
[d] Food Engineering Division, National Food Research Institute, National Agriculture and Food Research Organization, Tskuba, Japan

A R T I C L E I N F O

Article history:
Received 24 March 2015
Received in revised form
11 May 2015
Accepted 12 May 2015
Available online 21 May 2015

Keywords:
Fish
Freshness
K-value
Horse mackerel
Fluorescence spectroscopy
Excitation–emission matrix
Partial least square (PLS)

A B S T R A C T

The current study attempted to provide a convenient, non-invasive and time-saving method to estimate the freshness of intact horse mackerel (*Trachurus japonicus*) fish in a frozen state using autofluorescence spectroscopy in tandem with multivariate analysis of fluorescence excitation–emission matrices (EEM). The extracted fluorescence data from different freshness conditions were pretreated, masked and re-organized to resolve fish fluorescence spectra from overlapping signals and scattering profiles for detecting and characterizing freshness changes. The real freshness values of the examined fish samples were then traditionally determined by the hard chemical analysis using the high performance liquid chromatography (HPLC) method and expressed as *K*-values. The fluorescence EEM data and the real freshness values were modeled using partial least square (PLS) regression and a novel algorithm was proposed to identify the ideal combinations of excitation and emission wavelengths being used as perfect predictors. The results revealed that freshness of frozen fish could be accurately predicted with R^2 of 0.89 and root mean square error estimated by cross validation (RMSECV) of 9.66%. This work substantially demonstrated that the autofluorescence spectroscopy associated with the proposed technical approaches has a high potential in non-destructive sensing of fish freshness in the frozen state.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

As fresh fish are highly fragile, exceptionally perishable and susceptible to spoilage as well as undergo rapid oxidation and develop rancid/oxidized flavors throughout exposure to unfavorable conditions, extreme care must be practiced during handling, processing, transporting, packaging, and storage of these products to minimize these negative impacts. Immediately after harvesting, the fish flesh initiate alterations which led to the degradation of the perceived quality. Fresh fish is understood as being fish freshly caught or which has been chilled and stored for a short period at normal refrigeration temperature prior to purchase or use. Freshness could be considered one of the most important attributes used when assessing the quality of fishery products [1,2]. In Japan, freshness expression is the fundamental and crucial determinant of acceptability and pricing on the market because the valuable and prime fresh fish product is typically suitable to be eaten raw known as '*Sashimi*' and '*Sushi*' having a higher price than the same fish after several days in cold storage. To increase the transparency of the market, information related to the product's freshness should be announced to increase consumer confidence, and to protect producers through the enhancement of their productions.

With the increasing globalization of fishery product sales, processors, consumers, and regulatory officials have been seeking improved methods for determining freshness and quality. A number of instrumental techniques have been proposed to evaluate the state of fish freshness [3,4]. Chemical and biochemical methods for the evaluation of freshness are more reliable and accurate than sensory methods; since they eliminate personal opinions in quality scoring based on organoleptic changes occurred as fish storage time is extended. Among chemical methods, the concentrations of adenosine 5'-triphosphate (ATP) and its breakdown/degradation products, adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx), respectively, are used as indices of freshness and estimating future storage life in a wide variety of fish. The ratios among all or some of these nucleotide breakdown compounds are commonly used as

* Corresponding author. Tel.: +81 532 44 6765.
E-mail address: g.elmasry@vpac.cs.tut.ac.jp (G. ElMasry).

http://dx.doi.org/10.1016/j.talanta.2015.05.031
0039-9140/© 2015 Elsevier B.V. All rights reserved.

indicators of freshness quality [5]. The *K*-value, also called *K*-index, suggested by a Japanese research group in 1959 as an objective index of fish freshness [6] is defined as the ratio of non-phosphorylated ATP metabolites to the total ATP breakdown products. Also, the modified K_1 value, which represents the ratio of Hx to the total concentrations of IMP, HxR, and Hx and does not involve determination of ATP, ADP, or AMP [7] was used as freshness indicators. Both indices have been reported to have a potential as objective measures of freshness and reliable indicators for '*future*' storage life of fish [8]. In Japan, a *K*-value of 20% was suggested as a general threshold for fish to be consumed raw. With the abundance of worldwide trade in seafood products in recent years, researchers are paying increasing attention in seeking smart technical analyses because the traditional chemical or sensory methods are usually very time consuming and require expensive analytical laboratory equipment and reagents. Therefore, development of such rapid and reliable methods for the recognition of the incipient product quality and improving quality management system has become an imperative issue in research and industry.

Near-infrared (NIR) spectroscopy is one example of rapid and non-invasive methods that have been used efficaciously to quantify seafood composition [9,10] and as a tool for safety characterization [11], quality authentication [12–14], sensory evaluation [15] and freshness assessment [16] of various fishery products. On the other hand, fluorescence spectroscopy has shown potential for rapid, non-contact and nondestructive analysis of fishery and other food products for measuring concentrations down to one thousandth of what can be measured by normal absorption spectroscopy [17–19]. This technique depends on sensing the contents of naturally occurring fluorescent compounds (fluorophores) without extensive sample pretreatment and avoidance of handling dangerous reagents. The emission (luminescence) from these compounds is called intrinsic fluorescence or autofluorescence [20]. These fluorophores are a functional group in a molecule that absorbs energy, excites at a specific wavelength and re-emits energy at a different, specific wavelength(s). The amount of the emitted energy and the wavelength at which the energy emits depend on both the fluorophore itself and the chemical environment of the fluorophore. Hence, it is undeniable that autofluorescence from intact food products contains valuable information on the freshness status, composition and sensory properties of the analyzed fishery products. The fluorescence signals arising from such products are a combination of individual signals from different intrinsic fluorophores, at the same time influenced by the physicochemical environment of the food matrix such as temperature, pH and color [21]. The fluorescence intensity of a given fluorophore is a function of the fluorophore concentration and its specific excitation–emission spectral profiles. These profiles can be measured as excitation and emission spectra or as a complete excitation–emission matrix (EEM), also known as fluorescence landscapes [22]. Furthermore, the high sensitivity of photodetectors and the ability of monochromators or filters to resolve incident light from emitted light makes fluorescence a sensitive analytical procedure to detect trace quantities of one or more of these compounds simultaneously [23].

Within the last three decades, an increasing amount of research in using autofluorescence spectroscopy has been issued. The high performance sensitivity and specificity in combination with expeditiousness and non-destructive nature of fluorescence analysis makes fluorescence spectroscopy a potential choice as a screening method of food products, both in food production and in regulatory affairs. In this regard, it was quite useful to utilize this technique in identifying fish quality parameters for fresh and/or thawed samples [16]. Immediately after postmortem, several biochemical and enzymatic changes are triggered in seafood muscles, especially with improper storage and handling, leading to a faster rate of spoilage than mammalian muscles due to high water and free amino acid contents and lower content of connective tissue. Besides, once the fresh and un-fresh (aged) fish are getting frozen, they always look eventually the same and it would be rather difficult to differentiate them directly by naked eyes. The only way to discover the difference between fresh and un-fresh frozen fish is to let them to thaw and then assess their freshness statues either optically or destructively using the conventional wet chemical analyses. The challenge is to estimate the freshness status non-destructively when fish are in their frozen state without thawing. Controlling the procedure of freshness labeling is only possible if a rapid and reliable method exists, which allows distinguish between fresh and unfresh fish. The idea depended on the fact that freezing process preserves the intact tissue metabolic state without changes for a long time. In addition, very low temperature provides high fluorescence quantum yield of different fluorophores [24] which enables their characterization in the frozen states compared with room temperature [25]. Hence, changes occurred in the fluorescent-emitting molecules during degradation of aged fish before freezing process could be tracked using their fluorescence signals [26]. Accordingly, the main aim of this work was to estimate the original freshness conditions of frozen fish by tracking changes in their fluorescence behavior for the purpose to reach a better understanding of the main fluorophores that have a strong contribution to freshness properties. The study will also spot the light on using different chemometric techniques to analyze fluorescence spectra as well as developing a novel modeling algorithms to identify the suitable excitation–emission wavelength combinations sensible to freshness changes.

2. Materials and methods

2.1. Fish samples

The fresh horse mackerel (*Trachurus japonicus*) used in this study (average weight and length: 130.4 ± 10.6 g and 19.0 ± 0.6 mm, respectively) were purchased alive from a local fish market (Toyohashi, Japan) in May 2014. The fresh samples were harvested in the same day of purchase and dispatched to the laboratories of Toyohashi University of Technology packed in an insulated polystyrene box filled with ice. All samples were vacuum packed individually and four fish (prime fresh) were immediately frozen (d0), while the rest were stored in a cold chamber at a temperature commonly used in commercial and home refrigeration of 2–4 °C for up to 12 d from the time of purchase to stimulate different freshness conditions. Fluorescence measurements were then acquired for frozen samples of fresh fish (0d) as well as for samples previously stored for 1, 4, 7 and 12 d. Once the fluorescence spectra were acquired, the samples were repacked and kept frozen at −30 °C till the chemical determination of freshness (Fig. 1).

2.2. Acquisition of fluorescence spectra

The fluorescence fingerprint of each frozen fish was acquired using a fluorescence spectrometer F-7000 (Hitachi High-Technologies Ltd., Japan) supported with an external *Y*-type fibreoptic probe to measure fluorescence intensity directly inside the freezer (−30 °C) without transferring the samples inside the scanning compartment of the device. As shown in Fig. 1, each intact fish sample was positioned in the middle of the freezer and the optical fiber probe was positioned 2 mm above the sample to capture its fluorescence spectrum. Holding samples inside the freezer during fluorescence measurements helped in minimizing temperature changes of the samples and keeping them in their original freshness

Download English Version:

https://daneshyari.com/en/article/1241868

Download Persian Version:

https://daneshyari.com/article/1241868

Daneshyari.com